Supplementary Information for **Binding of heparin to defensin inspired peptides: Insights to antimicrobial inhibition**

Materials and Methods

Production of recombinant Defr1 1C^{v} 5S

A codon optimised Defr1 1C^{v} 5S gene was produced by recursive PCR using a series of overlapping oligonucleotide primers, and the resulting synthetic gene was subsequently inserted into the pET-31b expression vector (Novagen) using *AlwNI* restriction sites. The resulting construct, pET-31b/Defr1 1C^{5} 5S, contained the defensin gene downstream of a keto-steroid isomerase gene (*ksi*), and was used to express the KSI-defr1 1C^{5} 5S fusion protein in *E. coli* as insoluble inclusion bodies. The fidelity of plasmid was verified by DNA sequencing, before transformation of *E. coli* BL21(DE3) (Novagen). Cells were grown in 2YT growth media supplemented with ampicillin (100 μg/ml) and in shake flasks at 37 °C and 250rpm to OD_{600} = 0.3 before induction with 1.0 mM isopropyl-1-thio-β-D-galactopyranoside (IPTG). After a further 6 hours, cells were collected by centrifugation and the cell pellet was stored at 4 °C.

The cell pellet was resuspended in 5ml/ g wet weight resuspension buffer (50 mM Tris, 25% (w/v) Sucrose, 1 mM EDTA, 10 mM DDT, pH 8.0) before addition of 50 mg lysozyme and 5 units DNAse I. Lysis buffer (50 mM Tris, 1% (w/v) Triton-X100, 100 mM NaCl, 10 mM DDT, pH 8.0) was then added (5ml/ g wet weight) before the cell suspension was incubated at room temperature for 45 minutes. 700 ul EDTA (500 mM) was then added and the suspension was frozen and in liquid nitrogen and thawed at 37 °C. Subsequently, 500ul of $MgCl_2$ (500 mM) was added and the suspension was incubated at room temperature for 30 minutes before the crude inclusion bodies were

collected by centrifugation at 12,000*g* for 20 minutes at 4 °C. The inclusion bodies were then washed three times with wash buffer (50 mM Tris, 1% (w/v) Triton-X100, 100 mM NaCl, 1 mM DDT, 1 mM EDTA pH 8.0), until the fusion-protein was judged >90% pure by SDS-PAGE. Finally, the fusion protein was dissolved in a minimum quantity of solublization buffer (50 mM Tris, 6 M Guanidine, pH 8.0) and any insoluble debris was removed by centrifugation at 18,000*g* for 20 minutes at 4 °C. The fusion protein was then precipitated by addition of chilled water to dilute the guanidine to a final concentration of 1 M; before the purified fusion protein was collected by centrifugation at 12,000*g* for 20 minutes at 4 °C.

Chemical cleavage of the KSI-Defr1 1C^{v} 5S fusion protein and purification of Defr1 1C^{v} 5S.

The fusion protein was dissolved in a minimum quantity of 70 % (v/v) formic acid and was treated with 0.5 M cyanogen bromide. The reaction was performed under nitrogen at 22 °C and was allowed to proceed for 18 hours in the dark. After completion, the mixture was then evaporated by 2/3 under vacuum and freeze dried. The cleaved defensin was extracted from the mixture with 25 mM phosphate buffer pH 7.5. Finally, the defensin was purified by cation exchange chromatography using a Mono-S column attached to an AKTA-FPLC system (GE healthcare). SDS-PAGE analysis was used to verify that the McjA protein was >98% pure and LC-MS analysis revealed that the protein has a mass of 3724.3 Da (which is consistent with the theoretical value of 3723.9 Da).

The pure peptide was then subject to air oxidised in 10 mM phosphate buffer, pH 7.8 containing 10% DMSO for 24 hours. Oxidation was monitored by LC-MS and the resulting covalent dimer was found to have a mass of 7446.0 Da (calculated 7445.9 Da). Finally, the oxidised peptide was purified by cation exchange chromatography

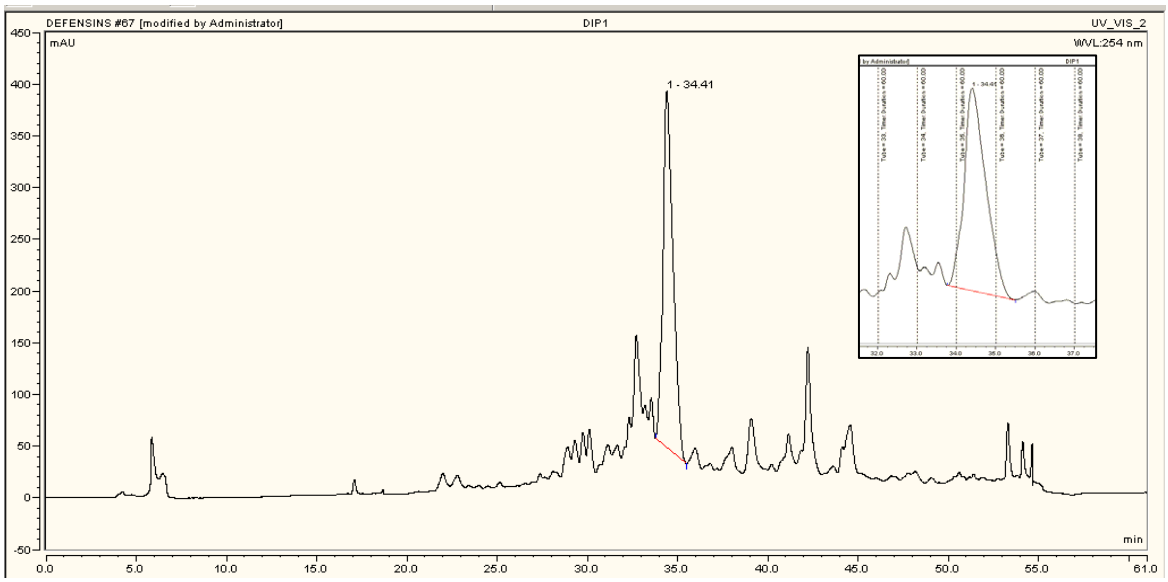
and the final peptide was stored at -20 °C in 0.01% acetic acid at a concentration of 1 mg/ml.

Peptide synthesis

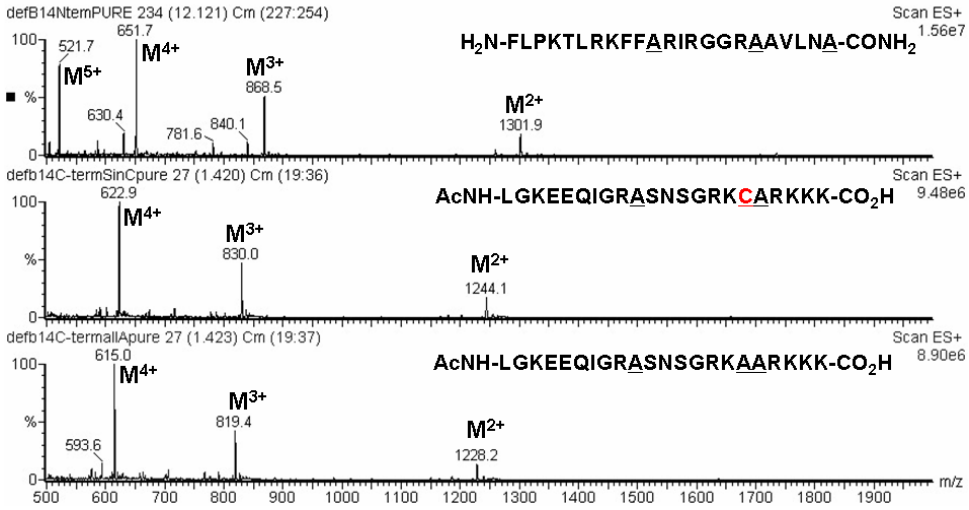
Defb14 0C, Defb14 1C^{v}, Defb14 1C^{v} 1-23, Defb14 1C^{v} Δ1-23, and Defb14 0C Δ1-23 peptides were made "inhouse" using automated peptide synthesis. This was carried out on an Applied Biosystems model 433A peptide synthesizer using Rink amide AM resin for peptide amides, pre-loaded NovaSyn®TGT resin for peptide acids and Fmoc amino acids from Novabiochem. LC- Mass spectra confirming identity and purity were obtained on a Micromass Quattro LC mass spectrometer. Semi-preparative HPLC was performed using a Phenomenex Luna C18 column and a gradient of 5-60 % acetonitrile (containing 0.1% TFA) over 45 minutes (flow rate of 3.0 mL/min). All other chemical reagents were obtained from Aldrich. Automated solid-phase peptide synthesis was carried out using the Fastmoc protocol on a 0.05 mmol scale using 0.5 mmol of each Fmoc amino acid per coupling reaction and HBTU/HOBt as coupling reagents. Coupling time was 0.5 h. Peptide products were cleaved from the resin with 95 % TFA, 2.5 % ethanedithiol, 2.5 % water for 3 h, the resin filtered-off, washed with TFA, and filtrate poured into diethylether (10 volumes). Following centrifugation (3000 rpm, 15 mins) the precipitate was re-suspended in ether (5 volumes) and re-centrifuged (3000 rpm, 15 mins). The crude peptides were dissolved in water and loaded directly onto a semi-preparative HPLC column. Peptide fractions were identified by mass spectrometry and lyophilized. Isolated yields for the DIP peptides was typically 16-23 mg (12.5-17.5 % yield). The yield for the synthesis of DefB146A was 80 mg (25 %).

Purification and Characterization of Defb14 1C^{v} 1-23, Defb14 1C^{v} Δ1-23, and

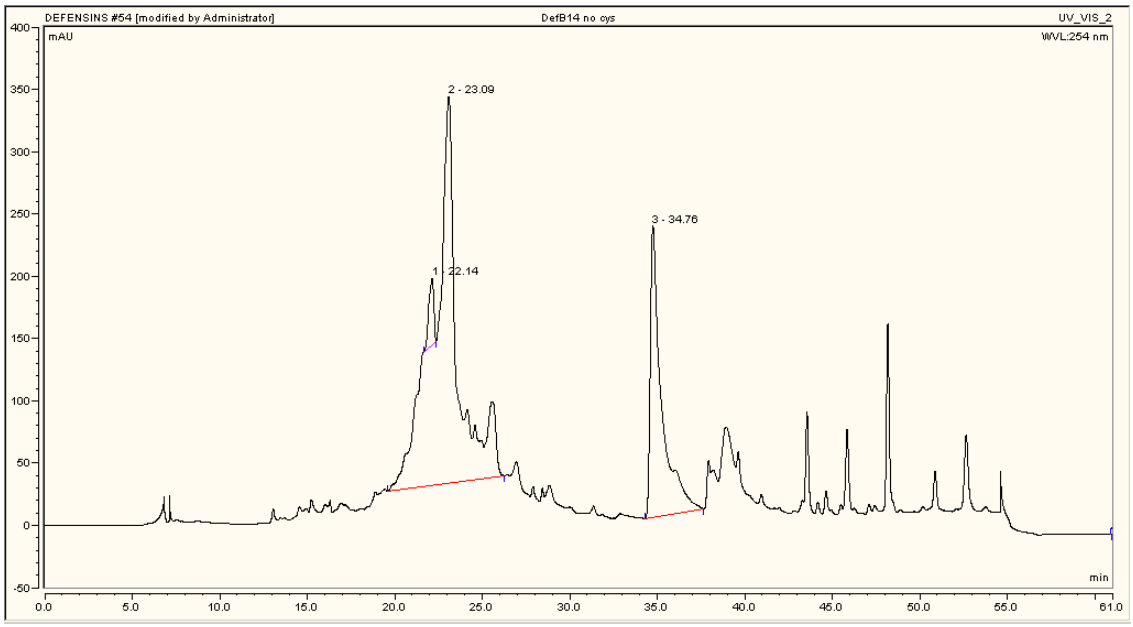
Defb14 0C Δ1-23**:**



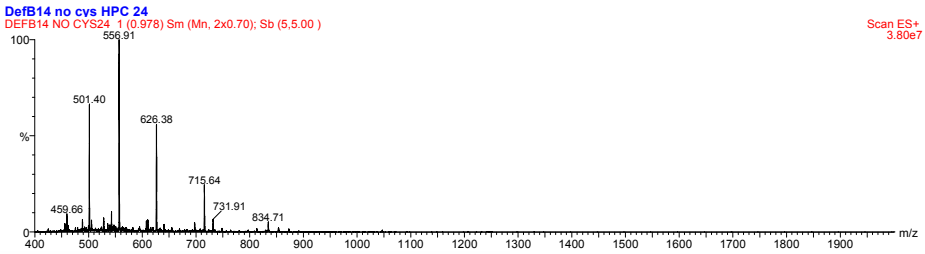
The contents of tube 35 were analyzed by mass spectrometry (below) and similar data were collected for Defb14 1C^{v} Δ1-23, and Defb14 0C Δ1-23:



HPLC purification of DefB14 0C



The peak at 23 minutes was collected and analyzed by mass spectrometry and found to have a mass consistent with that given by the sequence for DefB14:



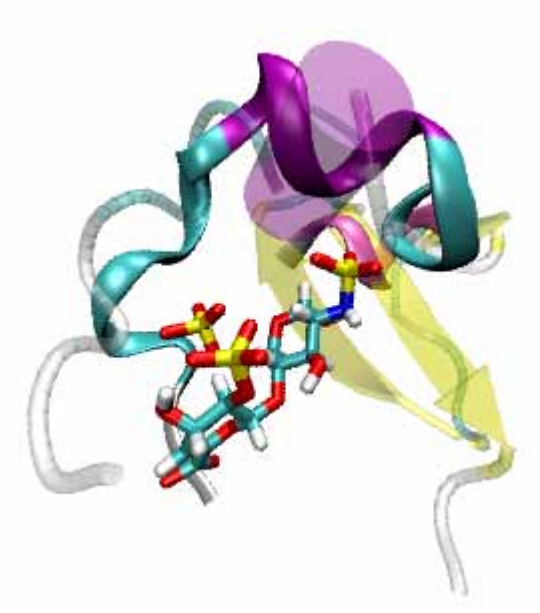


Figure 1S Low energy structure of the Defb14 1C^{v} 1-23:HDD complex, as shown in Figure 4A in the main text.. A ribbon structure represents the peptide; the licorice model represents the saccharide. Superimposed on this structure is the solution

structure of HBD-3[1] in cartoon form. Residues 1-20 in both structures have been aligned using VMD[2] and give an backbone RMSD of 5.2.

1. Schibli, D. J.; Hunter, H. N.; Aseyev, V.; Starner, T. D.; Wiencek, J. M.; McCray, P. B., Jr.; Tack, B. F.; Vogel, H. J., The solution structures of the human beta-defensins lead to a better understanding of the potent bactericidal activity of HBD3 against Staphylococcus aureus. *J Biol Chem* **2002,** 277, (10), 8279-89.
2. Humphrey, W.; Dalke, A.; Schulten, K., VMD: Visual Molecular Dynamics. *J. Mol. Graphics* **1996,** 14, 33.